NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF *p*-NITROANILINE

(CAS NO. 100-01-6)

IN B6C3F$_1$ MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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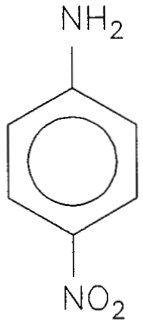
ABSTRACT



***p*-NITROANILINE**

CAS No. 100-01-6

Chemical Formula: $C_6H_6N_2O_2$ Molecular Weight: 138.12

p-Nitroaniline is an intermediate in the preparation of several azo dyes used for coloring consumer products. Toxicology and carcinogenicity studies were conducted by administering *p*-nitroaniline (>99% pure) in corn oil by gavage to groups of male and female B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, mouse lymphoma cells, and *Drosophila melanogaster*.

14-Day Studies

Groups of five male and five female B6C3F$_1$ mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 10, 30, 100, 300, or 1,000 mg/kg body weight 5 days per week for 2 weeks. All mice that received 1,000 mg/kg died from chemical-related toxicity by day 4 of the studies. Final mean body weights of mice receiving 300 mg/kg or less were similar to those of the controls. Hematology results were consistent with chemical-related methemoglobinemia and regenerative anemia. Methemoglobin concentrations in all groups of dosed mice were significantly higher than those in controls. Hematocrit values in mice that received 300 mg/kg and total erythrocyte counts in mice that received 100 or 300 mg/kg were significantly lower than those in controls. Reticulocyte counts in 300 mg/kg male mice and in 100 or 300 mg/kg females were significantly higher than controls. Heinz bodies were observed in erythrocytes of all 300 mg/kg mice and in two 100 mg/kg male mice. The absolute and relative spleen weights of 100 and 300 mg/kg mice were significantly greater than those of the controls. Hematopoiesis and pigment (hemosiderin) accumulation were observed in the splenic red pulp of males and females receiving 300 mg/kg; pigment (hemosiderin) accumulation in Kupffer cells of the liver was also seen in male mice at this dose level.

13-Week Studies

Groups of 20 male and 20 female B6C3F$_1$ mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 1, 3, 10, 30, or 100 mg/kg body weight 5 days per week for up to 13 weeks. Eight or nine mice in each group were evaluated at 7 weeks. There were no deaths associated with exposure to *p*-nitroaniline, and final mean body weights of dosed mice were similar to those of the controls. Hematologic and pathologic findings at 7 and 13 weeks were similar to those seen in the 14-day studies and occurred primarily in the 30 and 100 mg/kg groups. Methemoglobin concentrations were increased and hematocrit levels and erythrocyte counts were decreased relative to those of the controls. Heinz bodies were observed in

erythrocytes and nucleated erythrocytes and reticulocytes were increased in number.

Absolute and relative spleen weights of male and female mice receiving 30 and 100 mg/kg were significantly greater than those of controls at 7 and 13 weeks. Absolute and relative liver weights of female mice necropsied at 7 weeks were significantly greater in the 30 and 100 mg/kg groups; by 13 weeks, both absolute and relative liver weights were similar to control values. The incidence or severity of splenic hematopoiesis and pigmentation (hemosiderin) increased with dose at the 7-week interim evaluations and at the end of the studies. Pigment (hemosiderin) was also present in Kupffer cells of the liver in dosed male mice.

2-YEAR STUDIES

Groups of 70 male and 70 female B6C3F$_1$ mice received *p*-nitroaniline in corn oil by gavage at doses of 0, 3, 30, or 100 mg/kg body weight for 5 days per week for up to 103 weeks. The dose selection was based on the hematologic and pathologic findings of the 13-week studies. Nine or ten mice from each group were evaluated at 9 and 15 months for the presence of chemical-related lesions.

Body Weights, Clinical Findings, Survival, and Hematology

Mean body weights of male and female mice that received *p*-nitroaniline were similar to those of control mice throughout the 2-year studies. There were no clinical findings associated with chemical exposure, and survival of dosed mice was similar to that of controls. The hematology findings at the 9- and 15-month interim evaluations were similar to those in the 14-day and 13-week studies. The methemoglobin concentrations were significantly higher in all 30 or 100 mg/kg mice; sulfhemoglobin concentrations were significantly higher at 9 months in all 30 or 100 mg/kg female mice and at 15 months in 100 mg/kg females. Hematocrit and erythrocyte counts in 100 mg/kg mice were significantly lower than those in controls. By 9 months, reticulocyte counts were significantly higher in all 30 or 100 mg/kg mice. At 15 months, only the 100 mg/kg mice exhibited significantly higher reticulocyte counts.

Neoplasms and Nonneoplastic Lesions

Lesions related to the administration of *p*-nitroaniline occurred in the spleen, liver, and bone marrow, primarily in mice receiving 30 or 100 mg/kg; these were observed at the 9- and 15-month interim evaluations and at the end of the studies. There were increases in the incidence or severity of splenic congestion, hematopoiesis, pigment (hemosiderin) accumulation, Kupffer cell pigmentation in the liver, and bone marrow hypercellularity (hyperplasia).

The incidences of hemangiosarcoma of the liver (0 ppm, 0/50; 3 ppm, 1/50; 30 ppm, 2/50; 100 ppm, 4/50) and hemangioma or hemangiosarcoma (combined) at all sites (5/50, 3/50, 4/50, 10/50) were marginally increased in 100 mg/kg male mice. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly decreased (25/50, 26/50, 25/50, 13/50) in 100 mg/kg male mice.

GENETIC TOXICOLOGY

p-Nitroaniline is mutagenic *in vitro*. It was tested in two laboratories for induction of gene mutations in several strains of *Salmonella typhimurium*. Both studies showed positive results in strain TA98, with and without S9 activation; results were negative for all other strains. *p*-Nitroaniline was tested in two laboratories for induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. In the sister chromatid exchange study, one laboratory reported negative results without S9 and positive results with S9; the second laboratory reported equivocal results without S9 and negative results with S9. In the chromosomal aberrations study, both laboratories found positive results with S9. Without S9, one laboratory reported weakly positive results while the other reported negative results. *p*-Nitroaniline induced trifluorothymidine resistance in L5178Y mouse lymphoma cells in the absence of S9; no induction of trifluorothymidine resistance was noted with S9. In contrast to the positive results in the previous tests, *p*-nitroaniline did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered by feeding or injection to adult males or by feeding to larvae.

CONCLUSIONS

Under the conditions of these 2-year gavage studies there was *equivocal evidence of carcinogenic activity** of *p*-nitroaniline in male B6C3F$_1$ mice based on the increased incidences of hemangiosarcoma of the liver and hemangioma or hemangiosarcoma (combined) at all sites. There was *no evidence of carcinogenic activity* of *p*-nitroaniline in female B6C3F$_1$ mice receiving doses of 3, 30, or 100 mg/kg.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of *p*-Nitroaniline

	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses	0, 3, 30, or 100 mg/kg by corn oil gavage	0, 3, 30, or 100 mg/kg by corn oil gavage
Body weights	Similar to controls	Similar to controls
2-Year survival rates	33/50, 32/50, 36/50, 39/50	29/52, 41/50, 32/51, 32/51
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Uncertain findings	Liver: hemangiosarcoma (0/50, 1/50, 2/50, 4/50) All organs: hemangioma or hemangiosarcoma (5/50, 3/50, 4/50, 10/50)	None
Level of evidence of carcinogenic activity	Equivocal evidence	No evidence
Genetic toxicology		
Salmonella typhimurium gene mutation	Positive with and without S9 in strain TA98; Negative with and without S9 in strains TA100, TA1535, TA1537, and TA97	
Mouse lymphoma gene mutation	Negative with S9; positive without S9	
Sister chromatid exchanges		
Chinese hamster ovary cells *in vitro*:	Positive with S9; equivocal without S9	
Chromosomal aberrations		
Chinese hamster ovary cells *in vitro*:	Positive with S9; weakly positive without S9	
Sex-linked recessive lethal mutations		
Drosophila melanogaster:	Negative when administered by feed or injection	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on *p*-nitroaniline on November 21, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 21, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of *p*-nitroaniline received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of *p*-nitroaniline by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on neoplasms in male mice and nonneoplastic lesions in male and female mice. The proposed conclusions were *equivocal evidence of carcinogenic activity* in male B6C3F$_1$ mice and *no evidence of carcinogenic activity* in female B6C3F$_1$ mice.

Dr. M.J. van Zwieten, a principal reviewer, agreed with the conclusions. He thought there was insufficient discussion of the results of the 2-year study in rats recently reported in the literature. Dr. Irwin said the discussion of the rat study would be expanded. Dr. van Zwieten suggested that more discussion would be appropriate regarding selection of gavage administration when previous NTP studies of aniline and substituted anilines used the dietary route. Dr. Irwin said the compound was given by gavage because it was not stable in feed. Dr. van Zwieten said a brief histomorphological description of the vascular neoplasms observed would be useful in indicating the criteria used to distinguish benign from malignant lesions. Dr. Irwin agreed.

Dr. P.T. Bailey, the second principal reviewer, agreed with the conclusions. He questioned why 1,000 mg/kg was chosen as a dose level for the 14-day studies in view of the oral LD_{50} in mice cited as 750 mg/kg. Dr. Irwin commented that the top dose in the 14-day study is chosen to be sufficiently high enough to elicit a toxic response and, thus, may in some instances exceed the LD_{50}. Dr. Bailey wondered whether dietary administration would have been more akin to actual human exposure to the chemical.

Mr. L.S. Beliczky, the third principal reviewer, did not agree with the conclusions in male mice. He said that hemangioma or hemangiosarcoma (combined) at all sites showed a significant positive trend, and although incidences in the dosed groups were not significantly greater than controls by pairwise comparisons, the incidence of these neoplasms in the high-dose group (20%) exceeded the NTP historical control range (0% to 12%). Therefore, he thought the level of evidence in male mice should be *some evidence of carcinogenic activity*. Dr. Irwin said the level chosen was based on the fact that the neoplasms were only marginally increased in incidence and there was no comparable response in female mice. Mr. Beliczky commented that since these studies may have application to specific industries, the Production and Use section in the Introduction should be expanded to identify which type of industries manufacture and use the end products, among which are antioxidants and antiozonants. He believed that since 1978, NIOSH might have additional use and exposure data. Dr. Irwin asked Mr. Beliczky if he could obtain information about industries that produce these products.

Dr. L. Zeise questioned whether the maximum tolerated dose had been reached in the 2-year studies. Dr. Irwin replied that based on persistent anemia observed in 13-week studies, there was belief that some mortality was likely if 300 mg/kg were the top dose in the 2-year studies. Dr. S.L. Eustis, NIEHS, acknowledged that a higher top dose probably could have been tolerated, and a statement to that effect was added to the Discussion.

Dr. van Zwieten moved that the Technical Report on *p*-nitroaniline be accepted with the revisions discussed and with the conclusions as written for male mice, *equivocal evidence of carcinogenic activity*, and for female mice, *no evidence of carcinogenic activity*. Dr. Bailey seconded the motion, which was accepted by nine yes votes to one no vote (Mr. Beliczky).